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Fluorouracil prodrugs for the treatment of proliferative vitreoretinopathy: formulation in silicone oil and in vitro release of fluorouracil

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Abstract

Three new N_1-alkylcarbonyl-5-fluorouracil derivatives that are prodrugs of 5-fluorouracil (FU), one of them being a co-drug FU-retinoic acid (RA), were studied as potentially effective drugs against postsurgical proliferative vitreoretinopathy (PVR). The stability of N_1-octenoylFU (**3**), N_1-lauroylFU (**2**), and N_1-retinoylFU (**4**) in aqueous medium, their solubility in silicone oil (SiO), the kinetics of FU release in an in vitro system were determined. Compound **3** is very rapidly soluble in SiO. Its saturation concentration, reached after 6 h, is $233 \pm 13\,\mu g\,g^{-1}$ SiO. Compound **2** is not very soluble in SiO but its kinetic of solubilization is fast. Its saturation concentration, reached after 2 days, is $27 \pm 2\,\mu g\,g^{-1}$ SiO. Compound **4** is poorly soluble in SiO. A concentration plateau, with a mean value of $4\,\mu g\,g^{-1}$ SiO, is reached after 4 days. The addition in SiO of 5% of a perfluorinated perhydrogenated alkene greatly improves the solubilization of compound **4**. Two different types of FU release are observed. For compound **3**, the release is fast and is achieved after 1 day. For compounds **2** and **4**, the release is slower and is ended at 10 and 27 days, respectively. The solubility of the prodrugs in SiO is not correlated with their lipophilicity, whereas the release rate of FU decreased with increased lipophilicity of the prodrug. The most promising prodrug is compound **4** that slowly releases two active drugs (FU and RA) with a $t_{1/2\,release}$ of 5.8 days. It might be interesting for the treatment of PVR. However, an in vivo study on an animal model of PVR is necessary to prove the efficacy of this formulation and to study its toxicity.

Keywords: N_1-Alkylcarbonyl-5-fluorouracil prodrugs; N_1-Octenoyl-5-fluorouracil; N_1-Lauroyl-5-fluorouracil; N_1-Retinoyl-5-fluorouracil; Solubility in silicone oil; In vitro release of 5-fluorouracil; Proliferative vitreoretinopathy

1. Introduction

Despite continuing refinements in microsurgical instruments and vitreoretinal surgical techniques, proliferative vitreoretinopathy (PVR) is a major cause of failure of retinal detachment surgery. PVR is characterized by cellular proliferation creating membranes on both surfaces of the retina and within the vitreous, which then contract leading to the traction retinal detachment and failure of surgery. The precise pathogenic mechanisms involved in the formation of epiretinal membranes in PVR are not still completely understood. The contractile membranes are composed of retinal pigment epithelial (RPE) cells, glial cells, fibroblasts, and inflammatory cells (macrophages and lymphocytes). Among them, the main cells involved in the development of traction forces are the RPE

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cells (Nagasaki et al., 1998; Pastor et al., 2002; and references cited therein).

The prognosis for retinal detachments complicated with PVR has improved with the use of silicone oil (SiO) for prolonged retinal tamponade after surgery (Yamamoto and Takeuchi, 2000). Nevertheless, the risk of disease recurrence remains high for 2 months after the surgical act (Mietz and Heimann, 1995). Experimental and clinical studies suggested that pharmacological adjuvant therapy could decrease the proliferative disease process and so improve the success of surgery. Various treatments with corticosteroids (dexamethasone, triamcinolone), antiproliferative agents (daunomycin, aclacinomycin A, *N*,*N*-dimethyl-adriamycine, 5-fluorouracil (FU)), inhibitors of RPE cells proliferation and migration (retinoids) demonstrated an activity against the PVR process (Nagasaki et al., 1998).

Fluorouracil shows a good activity against animal models of PVR (Blumenkranz et al., 1982, 1984b; Stern et al., 1983a,b; Sunalp et al., 1984; Ophir, 1991; Ward et al., 1993). FU inhibits the spread and proliferation of RPE cells, but has no effect on cell migration (Chen et al., 1999). Due to the rapid clearance of the drug from the eye, multiple injections of FU are necessary to maintain a sustained level of the drug. Moreover, FU is insoluble in SiO, preventing the use of SiO as a vehicle of FU. A solution to these problems could be the elaboration of a FU prodrug soluble in SiO because of a long hydrocarbon chain grafted to the pyrimidine ring, which slowly releases the antiproliferative agent.

Retinoids are also effective on animal models of PVR (Doyle et al., 1992; Araiz et al., 1993; Giordano et al., 1993; Fekrat et al., 1995; Nakagawa et al., 1995; Takahashi et al., 1997; Veloso et al., 1997). They inhibit the proliferation and migration of RPE cells in vitro. Among them all-trans-retinoic acid (RA) has been shown to have the most inhibitory effect on RPE cell proliferation (Doyle et al., 1992).

Because FU and RA are both effective in two steps of the PVR process, it was believed that a synergistic approach to the prevention of PVR would be advantageous. We thus synthesized three prodrugs of FU, one of them being a co-drug FU–RA (Fig. 1). We evaluated their solubility in SiO and the kinetics of FU release in an in vitro system set up to mimic the eye in a very simplified way.

2. Materials and methods

2.1. Chemicals

FU (**1**), lauroic acid, octenoic acid, RA, and bromo-tris(dimethylamino)-phosphonium-hexafluoro-

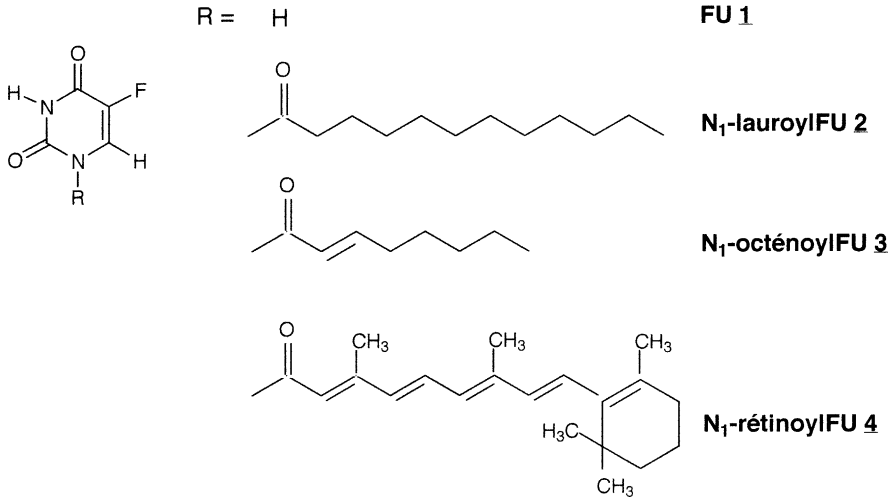

Fig. 1. Structures of 5-fluorouracil and prodrugs synthesized.

phosphate (BROP) were purchased from Sigma–Aldrich, France. Thirteen hundred centistokes purified SiO was a gift from Chauvin-Opsia, Toulouse, France, and the perfluorinated perhydrogenated alkene, $F_3C–(CF_2)_7–CH{=}CH–(CH_2)_9–CH_3$ (CF), from Elf Atochem, Pierre-Bénite, France. All other reagents were of analytical grade.

2.2. Equipment

UV spectra were obtained with a diode array Hewlett Packard 8452A spectrophotometer equipped with a thermostated cell compartment, using 1-cm quartz cuvettes. Fluorine-19 nuclear magnetic resonance (^{19}F NMR) spectra were obtained at 282.4 MHz on a Bruker AM 300 spectrometer.

2.3. Synthesis of FU prodrugs

N_1-Alkylcarbonyl prodrugs of FU (N_1-lauroylFU (**2**), N_1-octenoylFU (**3**), and N_1-retinoylFU (**4**)) (Fig. 1) were prepared as previously described by reacting FU with the appropriate acid in the presence of BROP as the coupling agent (Jolimaître et al., 1999). All compounds were characterized by ^{1}H, ^{13}C, and ^{19}F NMR spectroscopy, two-dimensional NMR spectroscopy (COSY H,H and HMQC H,C), UV spectrophotometry, mass spectrometry and, elemental analysis.

2.4. Lipophilicity

The lipophilicity of compounds **1–4** is given by the parameter log P, where P represents the partition coefficient in an octanol/water system. It was calculated by the software Chem Draw using lipophilic calculation tables set up by Viswanadhan et al. (1989).

2.5. Stability in aqueous medium

Hydrolysis rates were determined by UV spectrophotometry at 37.0 ± 0.1 °C in 0.1 M phosphate buffer (pH 7.4). The decrease of the absorbance at the λ_{max} of each prodrug was measured at 262, 274, and 400 nm for compounds **2**, **3**, and **4**, respectively. The synthesized products being insoluble in the buffer, a 5 mM stock solution of each prodrug was prepared in acetonitrile. Hydrolysis was initiated by adding 75 μl of the stock solution to 3 ml of phosphate buffer, prewarmed at 37 °C. Absorbances were recorded at appropriate time intervals and pseudo-first-order rate constants (k) were determined from linear plots of $\ln(A_t - A_\infty)$ versus time, where A_t and A_∞ are the absorbances at time t and ∞, respectively. The slopes ($-k$) of linear plots were determined by linear regression. Half-life time ($t_{1/2}$) was then obtained by the relation $t_{1/2} = \ln 2/k$. The experiments were done in triplicate for each prodrug.

2.6. Solubility in SiO

The kinetic of solubilization in SiO was studied for each prodrug. The SiO solutions containing the prodrugs were prepared as follows: 0.1 mmol of compound **3**, or 0.01 mmol of compounds **2** or **4** were placed in 50 g of SiO. These suspensions were stirred for several days at 25 °C. At appropriate times, between 6 h and 32 days, approximately 5 g of the suspension were removed, centrifuged for 30 min at 6000 rpm, then filtered on 0.45 μm Millipore filters. The amount of prodrug solubilized in SiO (SiO + P) was then determined by ^{19}F NMR spectroscopy and/or UV spectrophotometry.

For NMR measurements, 2.4 g of (SiO + P) was thoroughly mixed with 0.35 ml of $CDCl_3$. The solution was placed into a 10-mm diameter NMR tube. A coaxial capillary containing a solution of an external reference (sodium 4-fluorobenzoate (FBEN)) was then inserted in the tube. The apparent concentration of FBEN was previously measured against solutions of 5-fluorocytosine at known concentrations. ^{1}H-Decoupled ^{19}F NMR spectra were recorded in the following conditions: probe temperature, 25 °C; sweep width, 41,667 Hz; 32,768 data points zero-filled to 65,536; pulse width, 6 μs; pulse interval, 3.4 s; number of scans, 2000–6000; line broadening caused by exponential multiplication, 5 Hz. Under these recording conditions, fully relaxed spectra were obtained, as the signal intensities were unaffected when recording the spectra with a much longer repetition time (9.4 s). The chemical shifts (δ) were reported relative to the resonance peak of CF_3COOH (5% (w/v) aqueous solution) used as external chemical shift reference (δ = 0 ppm). Each prodrug gives a single ^{19}F NMR signal at a specific chemical shift (Table 1). The concentration of the prodrug in SiO was obtained by comparing its signal area to that of the external reference.

Table 1
^{19}F NMR chemical shifts (δ) and molar extinction coefficients (ε) of compounds **1**–**4**

Compound	^{19}F NMR δ (ppm)[a]	ε at λ_{max} ($l\,mol^{-1}\,cm^{-1}$)
1	−93.4 in D_2O	5300 or 5900 at 268 nm in phosphate buffer[b]
2	−85.9 in SiO/$CDCl_3$	10700 at 262 nm in SiO/$CHCl_3$
3	−86.0 in SiO/$CDCl_3$	12900 at 274 nm in SiO/$CHCl_3$
4	−87.0 in SiO/$CDCl_3$	22300 at 400 nm in SiO/$CHCl_3$

[a] Chemical shifts are relative to 5% (w/v) CF_3COOH aqueous solution.
[b] ε_{FU} is concentration-dependent: 5300 and 5900 in concentration ranges 4×10^{-5} to 3×10^{-4} M and 10^{-6} to 5×10^{-5} M, respectively (cf. Section 2.7).

For the UV measurement of the concentration of each prodrug dissolved in SiO, the molar extinction coefficient (ε) was first determined. As it was not possible to prepare solutions of known concentration in SiO, the calibration was done in a mixture of SiO and chloroform. The SiO/$CHCl_3$ ratio was 25/75 for compounds **2** and **4**, and 4/96 for compound **3** (Table 1). The absorbance of (SiO + P) was then measured in the same conditions at the λ_{max} of each prodrug (262, 274, and 400 nm for compounds **2**, **3**, and **4**, respectively) and the concentration calculated from the Beer–Lambert expression.

2.7. In vitro FU release study

Saturated solutions of each prodrug in SiO were prepared as indicated in the previous paragraph, and their concentrations determined after 2 days of stirring at 25 °C for compounds **2** and **3**, or 8 days for compound **4**.

For one release experiment, about 15 polypropylene tubes (volume 100 ml; diameter 3 cm) were used. Each tube contained 10 ml of phosphate buffer (0.1 M; pH 7.4) for compounds **2** and **3** or 2.5 ml for compound **4**, and 2.5 g of the saturated solution (SiO + P). A needle was placed in the tube. The tubes were closed and kept in an incubator at 37 °C for several days (Fig. 2). The tubes containing compound **4** were kept in the dark. At appropriate times, the whole aqueous phase from one tube was removed through the needle. The amount of FU in the aqueous phase was determined by UV spectrophotometry and ^{19}F NMR spectroscopy for compounds **2** and **3**, and by UV spectrophotometry only for

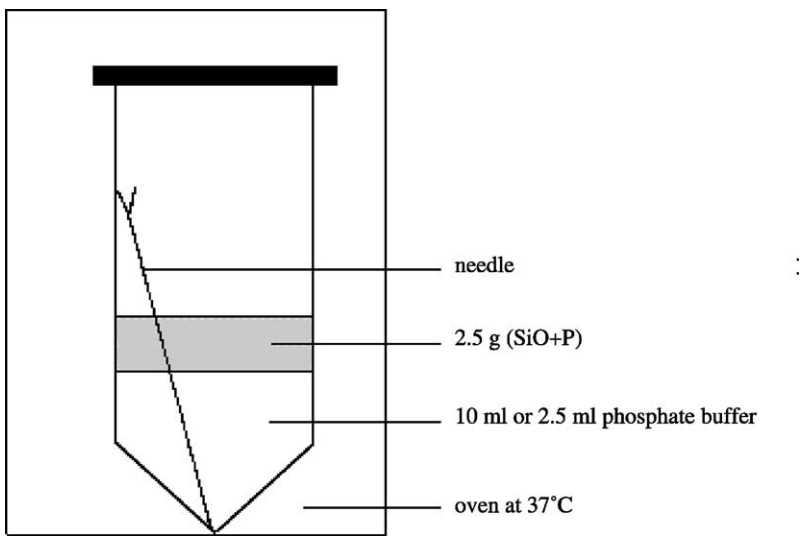

Fig. 2. Schematic representation of the in vitro release system.

compound **4** because of the too low concentration of FU.

UV quantification was carried out after the determination of ε_{FU} in phosphate buffer at 268 nm (FU λ_{max}). The concentration of FU was then obtained from the expression $C_{FU} = A/\varepsilon_{FU}$ l. ε_{FU} is concentration-dependent (it increases when concentration decreases), as already observed for 5′-deoxy-5-fluorouridine (Meynial et al., 1988). The calibration curves for FU were obtained from FU solutions whose concentrations were within the range expected for 10–100% release of FU from the three prodrugs in the experimental conditions described above ($\approx 2 \times 10^{-5}$ to 2×10^{-4} M for compound **3** or $\approx 10^{-6}$ to 2×10^{-5} M for compounds **2** and **4**). The relationships between FU concentrations (x) and absorbance (y) were linear in the concentration ranges $\approx 4 \times 10^{-5}$ to 3×10^{-4} M and $\approx 10^{-6}$ to 5×10^{-5} M. The regression equations were $y = 5300\ (\pm 110)\ x + 2 \times 10^{-3}$ ($\pm 4 \times 10^{-5}$), $r^2 = 0.9994$ (± 0.0004) and $y = 5900\ (\pm 120)\ x + 2 \times 10^{-2}$ ($\pm 4 \times 10^{-4}$), $r^2 = 0.9993$ (± 0.0004), respectively (S.D. between parentheses, mean of three experiments). The value of ε_{FU} was, thus, considered as 5300 for FU released from compound **3**, and 5900 for FU released from compounds **2** and **4**. The accuracy and precision determined with 3–5 assays for selected concentrations were better than 5%.

^{19}F NMR quantification was performed after concentrating the aqueous phase by lyophilization, in the same conditions as described above. FU gives a singlet at $\delta = -93.4$ ppm that was identified by addition of the authentic standard. FU concentration was determined by comparison of its signal area to that of the external reference FBEN. The precision of the method in the recording conditions described above was 5–10% depending on the concentration (Martino et al., 2000).

To ensure that the two analytical methods led to similar data, we compared the results obtained with UV and ^{19}F NMR for three experiments with compound **3**. Both methods gave close values (S.D. between the two methods usually <10%) (Table 2).

The ratio [(amount of FU measured in the aqueous phase)/(amount of FU introduced in SiO as prodrug)] × 100 gives the percentage of FU released. The release rate constants ($k_{release}$) were determined using the first-order simulation software fitting the equation $Y = Y_\infty\ (1 - \exp(-k_{release}t))$, where Y represents the percentage of FU released at time t and Y_∞ represents the maximum percentage of release (100%). The half-release time was then obtained by the relation $t_{1/2\,release} = \ln 2 / k_{release}$.

Table 2
Comparison of the data obtained by UV and ^{19}F NMR for measuring the percentage of FU released in the aqueous phase (phosphate buffer) from compound **3** solubilized in SiO

Time (h)	FU release (%)	
	UV[a,b]	^{19}F NMR[a,b]
0.5	23 ± 3	17 ± 1
1	33 ± 4	28 ± 3
2	44 ± 2	39 ± 2
3	49 ± 2	57 ± 5
4	56 ± 1	57 ± 1
6	65 ± 2	56 ± 4
10	82 ± 4	83 ± 1
18	91 ± 2	94 ± 2
25	95 ± 3	103 ± 3
43	98 ± 2	115 ± 4
48	100 ± 3	110 ± 2

[a] Mean of three measurements.
[b] A paired Student's t-test on UV and ^{19}F NMR data from each experiment were not significant ($P > 0.1$).

It was considered that the three FU prodrugs dissolved in SiO were not degraded at the start of the release studies for the following reasons. First, the signal of FU was never observed when SiO solutions were recorded with ^{19}F NMR. Second, to eliminate particles of FU that might have been formed as FU is insoluble in SiO, SiO solutions were filtered before dosing at the beginning of FU release experiments. Third, 100% of FU expected from the hydrolysis of the prodrugs were recovered in the aqueous phase at the end of release experiments.

3. Results

3.1. Lipophilicity

The calculated values of log P of FU and the prodrugs **2**–**4** are given in Table 3. The prodrugs are all much more lipophilic compared to parent FU, and the longer the carbonyl chain, the more lipophilic the compound.

Table 3
Lipophilicity (log *P*), rate constants (k_{aq}) and half-lives ($t_{1/2\,aq}$) of hydrolysis (phosphate buffer, pH 7.4, 37 °C) of compounds **1**–**4**

Compound	log *P*	k_{aq} (min^{-1})	$t_{1/2\,aq}$ (min)
1	−1.31		
2	3.05	$1.4 \times 10^{-1} \pm 7 \times 10^{-3}$	4.9 ± 0.3
3	1.36	$2.1 \times 10^{-2} \pm 2 \times 10^{-3}$	34 ± 3
4	3.59	$1.3 \times 10^{-2} \pm 1 \times 10^{-3}$	52 ± 4

3.2. Stability in aqueous medium

The prodrugs **2**–**4** were all found to hydrolyze quantitatively to FU in phosphate buffer solution at pH 7.4. The hydrolyses follow a pseudo-first-order kinetic, and the k_{aq} and $t_{1/2\,aq}$ values for each prodrug are reported in Table 3. Compound **2** with a long saturated chain hydrolyses very rapidly. In contrast, the prodrug **4** is the most stable compound. The stability of compound **3** is intermediate.

3.3. Solubility in SiO

The solubility of each prodrug in SiO has been studied as a function of time (Table 4). The solubilization profile for each prodrug is different (Fig. 3). Compound **3**, which is the most soluble, already reached its maximum concentration after 6 h. In contrast, the more lipophilic prodrugs **2** and **4** are less soluble. The maximum concentration of compound **2** was observed after 2 days. Compound **4** presents a plateau in its solubilization after 4 days.

The solubilization of compound **4** was also studied in a mixture of SiO and a perfluorinated and perhydrogenated compound $F_3C–(CF_2)_7–CH{=}CH–(CH_2)_9–CH_3$ (CF) readily soluble in SiO. We thought that the perhydrogenated part could help the solubilization of compound **4**. Two different concentrations of CF in SiO, 1 and 5%, were employed. The concentrations of compound **4** dissolved in the SiO/CF mixture are reported in Table 4. The solubilization profiles show that the addition of 1% CF slightly improved the solubilization in SiO (Fig. 3). On the other hand, the addition of 5% CF significantly increased the solubilization of compound **4** as its concentration has been multiplied by a factor >2.

In conclusion, compound **3** is very and rapidly soluble in SiO. Its saturation concentration, reached after 6 h, is 233 ± 13 μg g^{-1} SiO. Compound **2** is not very soluble in SiO but its kinetic of solubilization is fast. Its saturation concentration, reached after 2 days, is 27 ± 2 μg g^{-1} SiO. Compound **4** is poorly soluble in SiO. A concentration plateau, with a mean value of 4 μg g^{-1} SiO, is reached after 4 days. Moreover, the addition in SiO of 5% of a perfluorinated perhydrogenated alkene greatly improves the solubilization of compound **4**.

Table 4
Solubilization of compounds **2**–**4** in SiO and, for compound **4** only, in SiO containing 1 or 5% of $F_3C–(CF_2)_7–CH{=}CH–(CH_2)_9–CH_3$ (CF)

Time (day)	Compound **2**	Compound **3**	Compound **4**		
	Concentration[a] (μg g^{-1} SiO)		Concentration[b] (μg g^{-1} SiO) ($n = 3$)	Concentration[b] (μg g^{-1} SiO + 1% CF) ($n = 1$)	Concentration[b] (μg g^{-1} SiO + 5% CF) ($n = 3$)
	$n = 3$	$n = 4$[c]			
0.25		233 ± 13 ($n = 3$)			
0.5		259 ± 6 ($n = 3$)			
1	20 ± 2	227 ± 50 ($n = 6$)	1.9 ± 0.2	2.8	6.7 ± 2.2
1.5		265 ± 23			
2	27 ± 2	214 ± 32 ($n = 7$)	2.3 ± 0.2	4.3	7.0 ± 2.3
3	23 ± 2	215 ± 10	3.0 ± 0.007	4.4	6.7 ± 1.3
4	29 ± 5	209 ± 14	3.8 ± 1.1	4.5	7.5 ± 1.9
5	29 ± 1	214 ± 44	3.8 ± 0.5	5.1	7.0 ± 1.4
7	23 ± 7	205 ± 21	4.1 ± 0.9	5.7	
9	22 ± 6	214 ± 24	3.8 ± 0.4	5.4	9.0 ± 1.2
12		210 ± 42	4.2 ± 0.4	5.9	10.6 ± 2.6
15	24 ± 20	226 ± 38	5.2 ± 0.3	6.1	10.6 ± 0.4

[a] The values are the means of ^{19}F NMR and UV assays.
[b] The values were only obtained from UV analysis.
[c] Unless otherwise indicated.

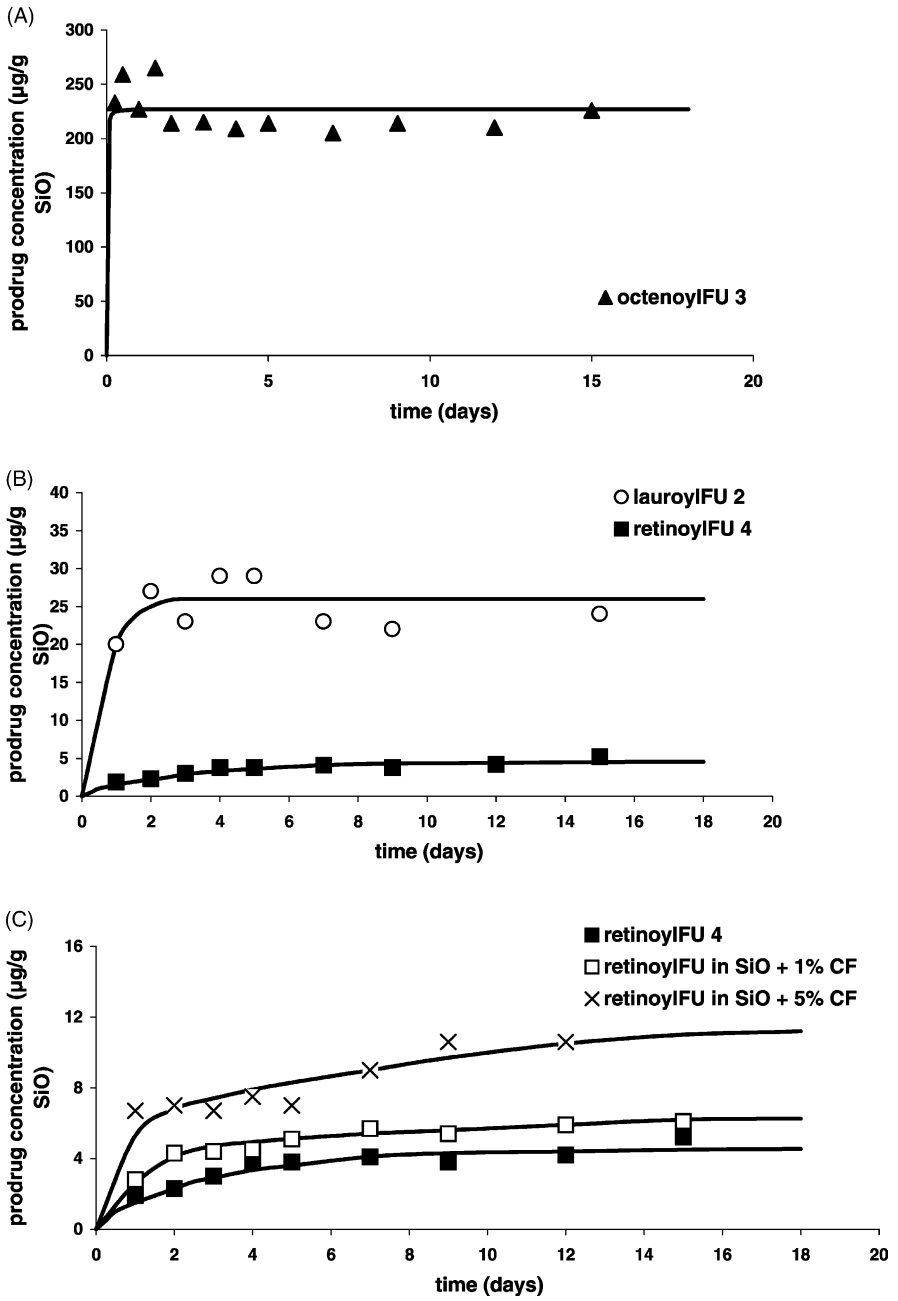

Fig. 3. Evolution with time of the solubilization of (A) N_1-octenoyl FU (**3**), (B) N_1-lauroylFU (**2**) and N_1-retinoylFU (**4**) in silicone oil, and (C) N_1-retinoylFU (**4**) in silicone oil alone or in silicone oil containing 1 or 5% of the alkene $F_3C–(CF_2)_7–CH{=}CH–(CH_2)_9–CH_3$ (CF).

3.4. In vitro FU release studies

The rate of FU release in aqueous medium was determined from saturated solutions of each prodrug in SiO. The amount of FU in the aqueous phase was, thus, quantified as a function of time (Table 5). A first order simulation software applied to the release of FU from the three prodrugs gave the curves shown in Fig. 4.

Table 5
Kinetic of FU release in an aqueous phase (phosphate buffer) from compounds **2**–**4** solubilized in SiO

Compound **2**		Compound **3**		Compound **4**	
Time (day)	% FU released[a] ($n = 3$)[b]	Time (h)	% FU released[a] ($n = 3$)[b]	Time (day)	% FU released[c] ($n = 3$)[b]
0.25	11 ± 1	0.5	20 ± 3 ($n = 4$)	1	21 ± 5 ($n = 4$)
0.5	13 ± 5	1	31 ± 3 ($n = 4$)	2	25 ± 8 ($n = 4$)
1	25 ± 1	2	42 ± 3	3	29 ± 7 ($n = 4$)
1.5	31 ± 6	3	53 ± 3	4	42 ± 6 ($n = 4$)
2	40 ± 2	4	57 ± 2	5	48 ± 9
2.5	46 ± 4	6	60 ± 3	6	46 ± 3
3	52 ± 3	10	82 ± 3	7	52 ± 9
3.5	57 ± 3	18	93 ± 1	9	62 ± 8
4	62 ± 1	25	99 ± 4	12	69 ± 5
4.5	65 ± 3	43	106 ± 5	20	83 ± 9
5	71 ± 3	48	105 ± 3	24	86 ± 14
6	77 ± 6	58	104 ± 6	27	97 ± 15
7	79 ± 4	72	99 ± 4	30	94 ± 9
8	89 ± 5	100	101 ± 8	57	110 ± 1
10	100 ± 0.3	121	99 ± 5		
12	102 ± 9	144	97 ± 3		
22	101 ($n = 1$)				
30	100 ($n = 1$)				

[a] The values are the means of ^{19}F NMR and UV assays.
[b] Unless otherwise indicated.
[c] The values were only obtained from UV analysis.

For compound **3**, the most soluble compound, the release is fast and is achieved after 1 day. For the two less soluble compounds, compounds **2** and **4**, the release is slower and is ended at 10 and 27 days, respectively.

The release of FU has also been studied for compound **4** solubilized in a mixture of SiO and 5% CF. Although only one experiment has been done, the release profile is the same than that observed when compound **4** is solubilized in SiO alone ($t_{1/2\,\text{release}}$ 5.4 days).

The rate constants of FU release from each prodrug are given in Table 6. The ratios $t_{1/2\,\text{release}}/t_{1/2\,\text{aq}}$ are different for compounds **2**–**4** (Tables 3 and 6).

The release of the corresponding acid was measured with ^{1}H NMR in only one experiment from each prodrug. For compound **3**, 43% of octenoic acid was found in the aqueous phase after 4 h of release experiment. For compound **2**, the amount of lauroic acid was 100% after 10 days. However, we were unable to detect RA in the aqueous phase from a release experiment with compound **4**. This could be explained by the low amount of compound **4** dissolved in SiO at the beginning of the experiment (≈4 μg g^{-1} SiO; Table 4) and the high solubility of RA in SiO (≈20 μg ml^{-1} SiO; Araiz et al., 1993).

Table 6
Rate constants (k_{release}) and half-lives ($t_{1/2\,\text{release}}$) of FU release from compounds **2**–**4**

Compound	k_{release}	$t_{1/2\,\text{release}}$
2	$2.5 \times 10^{-1} \pm 2 \times 10^{-2}$ per day	2.8 ± 0.3 days
3	$2.2 \times 10^{-1} \pm 1 \times 10^{-2}$ h^{-1}	3.2 ± 0.2 h
4	$1.2 \times 10^{-1} \pm 1 \times 10^{-2}$ per day	5.8 ± 0.5 days

4. Discussion

The aim of the study was to prepare a formulation allowing a sustained release of FU for a preventive treatment of PVR. Antiproliferative therapy with FU alone has been shown to be effective in the treatment of PVR in the animal model (Blumenkranz et al., 1982). However, due to the pharmacokinetics of the drug, multiple injections of FU are necessary to maintain a sustained level of the drug (Stern et al., 1983b). In the normal rabbit, the $t_{1/2}$ of FU after intravitreal injection is 7.7 h, whereas in aphakic vitrectomized

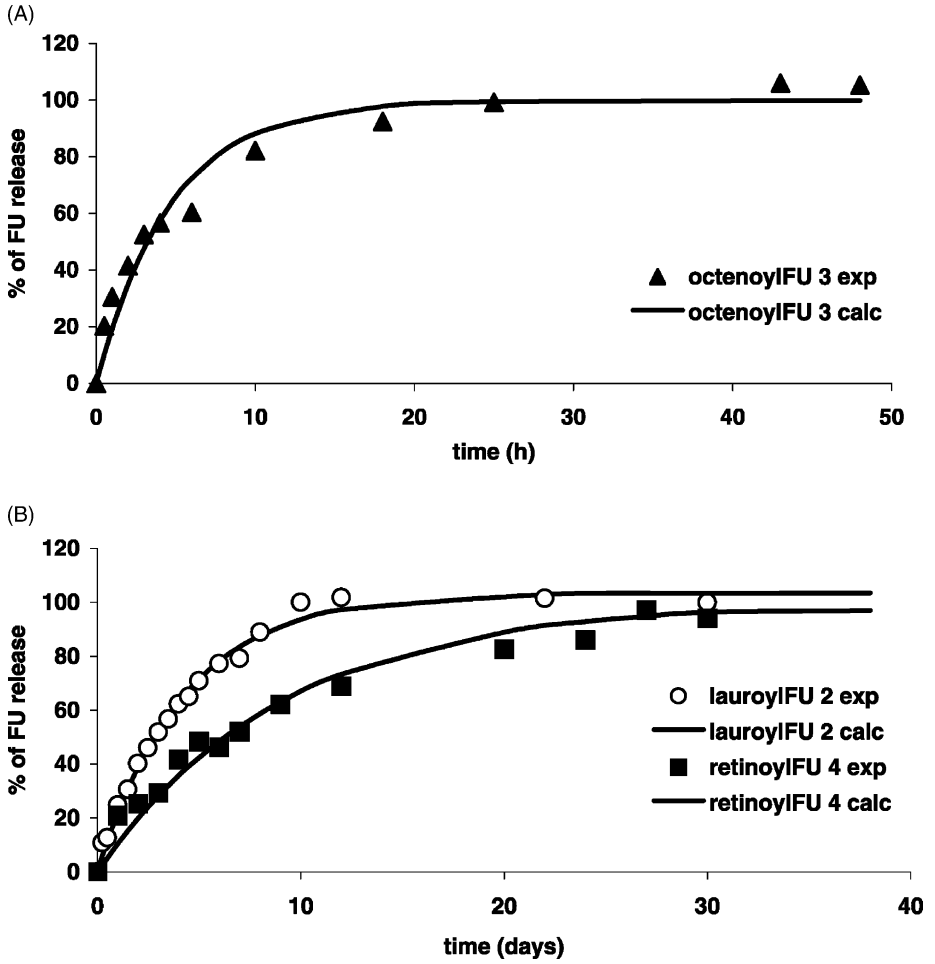

Fig. 4. Evolution with time of the percentage of FU released in the aqueous phase from (A) N_1-octenoylFU (**3**), (B) N_1-lauroylFU (**2**) and N_1-retinoylFU (**4**) dissolved in SiO.

animals, it is reduced to 3.2 h (Jarus et al., 1985). Repeated injections are associated with the risk for endophtalmitis and retinal detachment, as well as with inconvenience and discomfort of the patient. Moreover, the drug is toxic to the cornea and retina when administered in high dosage ($\geq$1 mg) (Binder et al., 1983; Stern et al., 1983a). Recently, a significant reduction in the incidence of postoperative PVR and in the reoperation rate resulting from PVR was demonstrated in patients receiving both FU and low molecular weight heparin (Asaria et al., 2001).

Obviously, PVR cannot develop immediately following any stimulus because implicated cells need time for dedifferentiation, migration, proliferation, synthesis of extracellular matrix, and subsequent contraction (Pastor, 1998). However, there is some discrepancy between data on the time of appearance of postoperative PVR. Mietz and Heimann (1995) reported that the average time interval between surgery and onset of PVR is 2 months (range 0.5–45 months). Aguirrebena et al. (1986) reported a retrospective study in which the average time to the development of postoperative PVR was shorter (about 1 month). In a prospective study on 223 retinal detachments, the symptoms of PVR were mostly detectable during the first postoperative month (Pastor et al., 2002). Mathis indicated a period of 6 weeks after the surgery (personal communication). However, the cells implicated

in the postoperative PVR process are activated by the surgical trauma. All these data suggest the possible need for a protracted treatment course that should also be efficient immediately after the surgery.

Some investigators have explored various methods for delivering intraocular FU over sustained periods of time such as liposomes, microspheres of biodegradable polymers, or biodegradable polymer rods (Nagasaki et al., 1998; Herrero-Vanrell and Refojo, 2001; and references cited therein). Other authors have chosen the use of a co-drug triamcinolone-FU or fluocinolone-FU compressed into a pellet that was inserted into the vitreous cavity. This device, which slowly liberated the two active principles, was effective in inhibiting the progression of PVR in a rabbit model and non-toxic. However, a relatively large surgical wound is necessary to insert the pellet (Berger et al., 1996; Yang et al., 1998; Perkins et al., 2000).

We have chosen the approach of synthesizing lipophilic FU prodrugs that can be solubilized in SiO. As RA has been shown to be effective on experimental model of PVR (Araiz et al., 1993; Nakagawa et al., 1995), one of them is a co-drug FU–RA, i.e. compound **4**, which, to our knowledge, has never been described. The two others are compounds **2** and **3** (Fig. 1). As expected, the lipophilicity of the prodrugs increased with the length of the carbonyl chain (Table 3). These data are in agreement with those reported in the literature for the N_1-alkoxycarbonyl prodrugs of FU (Steffansen et al., 1996).

In agreement with previous data on *N*-acyl or N_1-alkoxycarbonyl FU prodrugs (Buur and Bundgaard, 1984; Steffansen et al., 1996), the prodrugs **2**–**4** hydrolyzed quantitatively to FU. The hydrolysis of compound **2** that has a long saturated chain is very fast. Similar $t_{1/2}$ values were obtained by Beall et al. (1996) in their study of the hydrolyses of N_1-alkylcarbonyl derivatives of FU in 0.05 M phosphate buffer at pH 7.1 and 32 °C ($t_{1/2}$ comprised between 3 and 5 min for saturated linear chains of 1–7 carbon atoms). In contrast, the prodrug **4** is the most stable compound due to the strong delocalization on the retinoic chain that confers a particular stability to the N–C=O bond between the FU cycle and the alkyl chain. The stability of compound **3** is intermediate as the delocalization is weaker (Table 3).

There is no correlation between the lipophilicity of the prodrugs given by the value of log *P* (Table 3) and their solubility in SiO (Table 4). Compound **3**, the less lipophilic, is the most soluble in SiO, but it has the less bulky alkyl chain. Compounds **2** and **4**, with longer alkyl chains, are less soluble in SiO, although their lipophilicity is higher. The presence of an alkyl chain on the FU cycle, thus, allows the solubilization of the prodrug in SiO but the value of log *P* does not reflect the extent of solubilization. Actually, the solubilization process is not only based on classical hydrophile/hydrophobe interactions, and the steric hindrance of the alkyl chain might play an important role in this process. The alkyl chain of compound **4** is bulky and, in addition, the delocalization confers it a certain rigidity, which hinders the solubilization of the prodrug in SiO.

The release studies were performed from saturated solutions of each prodrug in SiO. At the interface, the prodrugs solubilized in SiO hydrolyzed into FU and the corresponding acid, which then went into the aqueous phase. The release of FU from the three prodrugs led to the curves shown in Fig. 4. The correlation between the experimental points and the simulated curve is good but not perfect. Two facts can explain the discrepancy. First, each experimental point is independent. Second, the kinetic is not simple as it is the result of at least three phenomenons: (i) the transport of the prodrug from the SiO phase to the interface; (ii) its hydrolysis at the interface; and (iii) the transport of FU from the interface to the aqueous phase. Each step has its own kinetic but, globally, the kinetic gets close to a first-order one.

Two different types of FU release are observed. The release is fast for compound **3**, the most soluble compound, and slower for the two less soluble compounds, compounds **2** and **4**. These data show that the release rate of FU decreased with increased lipophilicity of the prodrug, as already reported for N_1-alkoxycarbonyl prodrugs of FU dissolved or suspended in SiO (Steffansen et al., 1996). Moreover, as the ratios $t_{1/2\,\mathrm{release}}/t_{1/2\,\mathrm{aq}}$ for compounds **2**–**4** are different (Tables 3 and 6), the hydrolysis rate is not the limiting step of the release process. So, the rate of FU release in aqueous medium ($\boldsymbol{3} \gg \boldsymbol{2} > \boldsymbol{4}$) from the prodrugs dissolved in SiO is a function of their solubility in SiO ($\boldsymbol{3} \gg \boldsymbol{2} \gg \boldsymbol{4}$) but not of their hydrolysis rate in phosphate buffer ($\boldsymbol{2} \gg \boldsymbol{3} > \boldsymbol{4}$). The release rate of FU seems to be dependent on the concentration of the prodrug at the interface SiO/water.

Stern et al. (1983b) showed in an animal model after vitrectomy that it was necessary to give repeated injections of 0.5 mg of FU every 24 h for 7 days to achieve a non-toxic, yet clinically significant, effect because of rapid clearance of the drug from the eye. On the other hand, a therapeutic effect of a FU-containing implant was associated with sustained intravitreal concentrations of FU between 2.2 and 6.7 $\mu g\,ml^{-1}$ of vitreous fluid for 14 days (Rubsamen et al., 1994). Moreover, the FU concentration that has been reported to produce 50% inhibition of cell growth for fibroblast was 0.3 $\mu g\,ml^{-1}$ (Blumenkranz et al., 1984a). Even if extrapolation from in vitro studies must be done with extreme cautious, it seems that compound **3** would release a too high amount of FU in a too short period of time. Indeed, its saturation concentration is ≈230 $\mu g\,g^{-1}$ SiO (Table 4) and the $t_{1/2\,release}$ of FU from this prodrug is ≈3 h (Table 6). So, this compound is probably not a suitable prodrug for preventing postoperative PVR. Its only advantage compared with FU itself is its high solubility in SiO. On the other hand, compound **2** whose concentration at saturation is ≈25 $\mu g\,g^{-1}$ SiO (Table 4) leads to a FU $t_{1/2\,release}$ of 2.8 days with 100% of FU released after 10 days (Table 6; Fig. 4B). So, this compound should be considered for subsequent in vivo efficacy and toxicity studies. The co-drug compound **4** should be the most interesting compound as its $t_{1/2\,release}$ (5.8 days) is twice that of compound **2** and 100% of FU are released after 25 days (Table 6; Fig. 4B). Its solubility in SiO is rather low (concentration at saturation ≈4 $\mu g\,g^{-1}$ SiO) but it gives, after hydrolysis, two active drugs that could be more effective than treatment with either drug alone. Araiz et al. (1993) demonstrated that SiO containing 5 $\mu g\,g^{-1}$ of RA limits PVR development in an animal model, while no activity was observed for a RA concentration of 2 $\mu g\,g^{-1}$. No data were provided between these two concentrations. Even if our attempt to assay RA in the aqueous phase was unsuccessful, we could hypothesize that the amount of RA released from compound **4** could be superior to the inactive concentration found by Araiz et al. (1993) as 4 $\mu g\,g^{-1}$ of N_1-retinoylFU in SiO corresponds to ≈3 $\mu g\,g^{-1}$ of RA in SiO. Another interesting fact is that the solubility of compound **4** in SiO is strongly improved in the presence of a perfluorinated perhydrogenated alkene (Table 4).

In conclusion, the behaviour of three new N_1-alkylcarbonylFU prodrugs has been studied in an aqueous phase, an oily phase, and a biphasic system. The most promising prodrug is compound **4** that slowly releases two active drugs (FU and RA) with a $t_{1/2\,release}$ of 5.8 days in a biphasic system (phosphate buffer/SiO). This prodrug might be interesting for the treatment of PVR. However, an in vivo study on an animal model of PVR is necessary to prove the efficacy of this formulation and to study its toxicity.

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